MODULATION OF IgE RECEPTOR CELL SURFACE EXPRESSION

Government Support

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Field of the Invention

The invention relates to methods and related compositions for modulating cell surface expression of the high affinity receptor for immunoglobulin E, the FcεRI receptor. The invention also relates to methods and related compositions for the treatment and/or prevention of conditions mediated by IgE such as allergic conditions.

Background of the Invention

The FcεRI complex is the high affinity cell surface receptor for the Fc region of antigen specific immunoglobulin E (IgE) molecules. FcεRI is multimeric and is a member of a family of related antigen/Fc receptors which have conserved structural features and which exhibit similar functional activities in initiating intracellular signaling cascades. In humans, FcεRI controls the activation of mast cells and basophils, and participates in IgE mediated antigen presentation. Multivalent antigens bind and crosslink IgE molecules held at the cell surface by FcεRI. Receptor aggregation induces multiple signaling pathways that control diverse effector responses, including secretion of allergic mediators and the induction of cytokine gene transcription (such as IL-4, IL-6, TNFα and GM-CSF). FcεRI therefore is central to the induction and maintenance of an allergic response and physiologically may confer protection in parasitic infections.

It is a conserved feature of antigen receptors that they are multimeric, and that their individual subunits perform different functions. In the case of FcεRI, the receptor is composed of three distinct polypeptides. The α chain (FcεRIα) binds the Fc portion of IgE with high affinity, and the β chain (FcεRIβ) has four transmembrane domains between amino- and carboxyl-terminal cytoplasmic tails. A homodimer of two disulfide linked γ chains (FcεRIγ) completes the tetrameric structure. In humans, the tetrameric structure is not obligatory, and an alternate $\alpha\gamma_2$ trimer is present. In terms of devolution of function, the α chain contains two immunoglobulin type domains, D1 and D2, that mediate binding to IgE.

The β and γ chains contain conserved Immunoreceptor Tyrosine-based Activation Motifs (ITAM) in their cytoplasmic tails (2, 3). These motifs reportedly are phosphoacceptors, through which the receptor subunits interact with signaling proteins.

The events that control mast cell activation via FcεRI are sequential. First, IgE binds via its Fc fragment to the FcεRIα chain. Second, IgE molecules are cross-linked by multivalent antigen, causing aggregation of α chains in the plane of the plasma membrane. Third, information concerning productive α chain aggregation is transmitted to the βγ signaling subunits, via an unknown mechanism. The resulting initiation of intracellular signaling pathways controls downstream events such as allergic mediator production and cytokine gene transcription. These downstream signaling events are extensively reviewed in Kinet, J-P., et al, (1), and Turner, H.K. & Kinet, J-P. (4).

Antigenic crosslinking of the FcεRI initiates a chain of phosphate transfer events within the receptor microenvironment. The β and γ chains of the FcεRI contain ITAMs, where the tyrosine residues are phosphoacceptor sites for the action of receptor-associated protein tyrosine kinases (PTKs) (12, 13). Phospho-ITAMs link receptor and signal transduction cascades. In the FcεRI context, the β and γ ITAMs have slightly different structures and serve distinct functions. There are two species of FcεRI associated PTK; the *src* family kinase Lyn and the p72 Syk kinase. The former is found associated with FcεRIβ, the latter is able to bind β and γ but has higher affinity for interaction with γ.

Several studies report on the immediate events following FcεRI aggregation (1, 14), in which: 1) *The β and γ chains act cooperatively.* Both *in vitro* and genetic reconstitution studies illustrate this point. Reconstitution of FcεRI deficient mast cell lines show that mutation of the two canonical tyrosines in the β ITAM abolish activation dependent phosphorylation of both the β and the γ ITAMs, suggesting that the phosphorylation of the former has bearing on the status of the latter (15). Moreover, while $Lyn^{-/-}$ mast cells exhibit no β or γ phosphorylation, $Syk^{-/-}$ cells have intact β and γ phosphorylation but still lack downstream signaling events (16, 17, 18). 2*) Lyn binds to β under resting conditions.* An obvious candidate for the mediation of this interaction is the Lyn SH2 domain, since β is slightly tyrosine phosphorylated under resting conditions. However, others have reported that the 'unique' (SH4 containing) domain of Lyn interacts with FcεRIβ (19). 3) *Active Lyn phosphorylates the β and γ ITAMs.* Upon receptor aggregation, Lyn is activated and its catalytic activity becomes directed toward the β and γ ITAMs. Syk is then recruited to the

receptor γ chain via one of the tandem SH2 domains in the kinase. An important feature is that Lyn may transphosphorylate ITAM in other receptor complexes. 4) *Syk binding to FcεRIγ leads to Lyn-dependent tyrosine phosphorylation and activation of the kinase*. This step finally potentiates the productive interaction of active Syk with its many targets. In summary, aggregation leads to *Lyn*-dependent ITAM phosphorylation.

Rodent FcεRI receptor complexes have an obligatory tetrameric $\alpha\beta\gamma_2$ structure. In humans, both $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ complexes are observed at the cell surface. Rodent FcεRI receptor complexes are confined to the surface of mast cells and basophils. In humans, however, it is now recognized that there is a far wider distribution of FcεRI. On the mast cell and basophil surface there is a mixture of $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ complexes, while monocytes, Langerhans cells, eosinophils and dendritic cells express surface $\alpha\gamma_2$. Finally, in rodent but not human, there is crosstalk between IgE and IgG mediated cellular activation. In the rodent, the IgE Fc region can bind to two classes of low affinity FcγR, activatory and inhibitory isotypes. Both of these are expressed on mast cells and so there is a route by which IgE or IgG immune complexes may regulate mast cell function independently of FcεRI.

FcεRI receptor complexes are first assembled in the ER. Here, nascent α, β and γ chains are thought to interact non-covalently. Only the trafficking of α chains to the cell surface has been monitored extensively. In the ER, the α chain reportedly is core-glycosylated. During trafficking through the Golgi apparatus, the core, high-mannose glycosylations of α are replaced by complex sugar, terminal glycosylations. This difference can be exploited experimentally since the latter are insensitive to the action of Endoglycosidase H. The reason for this two stage glycosylation may be that both during biosynthesis and at the cell surface, it is important that α chains do not aggregate in the absence of antigen. *In vitro*, de-glycosylated FcεRIα aggregates spontaneously without antigen. The glycosylation of potential α-α interaction surfaces prevents premature aggregation and permits interaction with the synthetic machinery. Later, terminal glycosylation does permit aggregation by a multivalent antigen. Mature FcεRIα glycosylation does not extend to the top surface of the molecule, where the IgE interaction is proposed to take place.

Summary of the Invention

The invention is based, in part, on the discovery that an FcεRIβ chain variant modulates the expression of an FcεRI receptor (the high affinity receptor for IgE) in cells.

Accordingly, the invention provides methods and compositions for modulating cell surface expression of an FcεRI receptor in a cell (e.g., contained in a tissue or a subject) which expresses an FcεRI receptor.

More specifically, the invention is based, in part, on the discovery that expression of a variant of one of the constituent FcεRI receptor chains (e.g., an FcεRIβ chain variant, an FcεRIα chain variant, or an FcεRIγ chain variant) in a cell that expresses a wild type FcεRI receptor, results in the abrogation or decrease of FcεRI receptor cell surface expression. As a result, activity usually associated with such a receptor and mediation of IgE signals are also decreased or abolished. The invention, therefore, is useful whenever it is desirable to modulate such activity, for example, in the treatment of conditions mediated by IgE. Thus, the invention also provides methods and related compositions for identifying pharmacological agents useful in the treatment of such conditions.

According to one aspect of the invention, a method for inhibiting expression of an FcεRI receptor in a cell, is provided. The method involves contacting a cell expressing (i.e., a cell expressing or capable of expressing), an FcεRI receptor with an FcεRIβ chain variant in an effective amount to inhibit expression of the FcεRI receptor in the cell. In some embodiments, the FcεRIβ chain variant is an isolated nucleic acid molecule that inhibits expression of an FcεRI receptor in the cell. In preferred embodiments, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3. In certain embodiments, the FcεRIβ chain variant is an isolated peptide molecule that inhibits expression of an FcεRI receptor in the cell. In preferred embodiments, the isolated peptide molecule comprises the nucleotide sequence of SEQ ID NO:4. The contacting can occur *in vitro* or *in vivo*.

According to another aspect of the invention, a method for inhibiting expression of an FcεRI receptor in a subject to treat a condition mediated by IgE, is provided. The method involves administering to a subject in need of such treatment an FcεRIβ chain variant in an effective amount to inhibit FcεRI receptor expression in a cell of the subject. In one embodiment, the condition mediated by IgE is an allergic condition. In preferred embodiments the allergic condition is atopy, rhinitis, conjunctivitis, anaphylaxis, urticaria, or angioedema. In a certain embodiment, conditions mediated by IgE are IgE-dependent late phase reactions. In some embodiments, the FcεRIβ chain variant is an isolated nucleic acid molecule that inhibits expression of an FcεRI receptor in the cell. In preferred embodiments, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3. In

certain embodiments, the FcεRIβ chain variant is an isolated peptide molecule that inhibits expression of an FcεRI receptor in the cell. In preferred embodiments, the isolated peptide molecule comprises the amino acid sequence of SEQ ID NO:4. Any of the foregoing embodiments can further comprise co-administering to the subject an anti-allergic (anti-atopic) agent other than an FcεRIβ chain variant.

According to a further aspect of the invention, a method of screening for FcεRI receptor expression modulating agents, is also provided. The method involves (a) contacting a putative FcεRI receptor expression modulating agent with cell expressing (i.e., a cell expressing or capable of expressing) an FcεRI receptor, (b) measuring FcεRI receptor expression by the cell, and (c) determining whether FcεRI receptor expression by the cell is altered compared to FcεRI receptor expression by a control cell, wherein the control cell is contacted with an FcεRIβ chain variant. In some embodiments, the FcεRIβ chain variant is an endogenous nucleic acid molecule of the cell. In another embodiment the FcεRIβ chain variant is a heterologous nucleic acid molecule of the cell. In a preferred embodiment, the FcεRIβ chain variant comprises the nucleotide sequence of SEQ ID NO:3. In certain embodiments, measuring FcεRI receptor expression by the cell comprises using an anti-FcεRI chain-specific antibody.

According to another aspect of the invention, a method of screening for FcεRIβ chain variant expression modulating agents, is provided. The method involves (a) contacting a putative FcεRIβ chain variant expression modulating agent with a test cell expressing (i.e., a cell expressing or capable of expressing) an FcεRIβ chain variant, (b) measuring FcεRIβ chain variant expression by the cell, and (c) determining whether FcεRIβ chain variant expression by the cell is altered, compared to a control cell expressing an FcεRIβ chain variant in the absence of a putative FcεRIβ chain variant expression modulating agent. In certain embodiments the control cell expresses an FcεRIβ chain variant identical to the FcεRIβ chain variant expressed by the test cell. In some embodiments, measuring FcεRIβ chain variant expression by the cell comprises using reverse transcription-polymerase chain reaction (RT-PCR).

According to yet another aspect of the invention, a method for inhibiting expression of an FcεRIα chain in a cell, is provided. The method involves contacting a cell expressing (i.e., a cell expressing or capable of expressing), an FcεRIα chain with an FcεRIβ chain variant in an effective amount to inhibit expression of the FcεRIα chain in the cell. In some

embodiments, the FcεRIβ chain variant is an isolated nucleic acid molecule that inhibits expression of an FcεRIα chain. In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:3. The contacting can occur *in vitro* or *in vivo*.

According to a further aspect of the invention, a method for determining whether a subject has a condition mediated by IgE or a predisposition thereto, is provided. The method involves determining FcεRIβ chain variant expression in a subject suspected of having a condition mediated by IgE or a predisposition thereto, and comparing the FcεRIβ chain variant expression to a control. Lower levels of FcεRIβ chain variant expression in the subject as compared to the control are indicative for the presence of, or a predisposition to, a condition mediated by IgE in the subject. In some embodiments, FcεRIβ chain variant expression is mRNA expression. In certain embodiments, FcεRIβ chain variant expression is peptide expression.

These and other aspects of the invention, as well as various advantages and utilities, will be more apparent with reference to the drawings and the detailed description of the preferred embodiments.

Brief Description of the drawings

Figure 1. Diagrammatic sketches of: **A.** The domain structure of WT (wild type) and βT (variant) FcεRIβ chains; and **B.** Predicted topologies of WT and βT FcεRIβ chains.

Figure 2. Graph depicting levels of surface FcεRIα polypeptide chains in U937 cells stably transfected with αβTγ, αγ, and αβγ FcεRIβ isoforms. Each point represents a different clone.

Figure 3. Graph depicting FcεRI receptor cell surface expression in U937 αβγ (WT) FcεRI stable transfectants, transiently retransfected with either control or βT FcεRIβ cDNA.

Brief Description of the Sequences

SEQ ID NO:1 is the wild type (WT) human FcεRIβ chain cDNA sequence.

SEQ ID NO:2 is the wild type (WT) human FcεRIβ chain polypeptide sequence encoded by the cDNA sequence set forth in SEQ ID NO: 1.

SEQ ID NO:3 is the variant (βT or FcεRIβT) FcεRIβ chain cDNA sequence.

SEQ ID NO:4 is the variant (βT or FcεRIβT) FcεRIβ chain polypeptide sequence encoded by the cDNA sequence set forth in SEQ ID NO: 3.

SEQ ID NO:5 is a sense PCR primer from exon 2 of FcεRIβ.

SEQ ID NO:6 is an antisense PCR primer from exon 7 of FcεRIβ.

SEQ ID NO:7 is the wild type (WT) human FcεRIα chain cDNA sequence.

SEQ ID NO:8 is the wild type (WT) human FcεRIγ chain cDNA sequence.

SEQ ID NO:9 is the wild type (WT) mouse FcεRIβ chain cDNA sequence.

Detailed Description of the Invention

The invention is based, in part, on the discovery that a variant form of the β chain (FcεRIβ) of the FcεRI receptor, FcεRIβT (SEQ ID NO:4), inhibits FcεRI receptor expression in a cell expressing (i.e., a cell expressing or capable of expressing), an FcεRI receptor. More specifically, we have discovered that the FcεRIβ chain variant prevents maturation (i.e., post-translational modifications) of FcεRI receptor chain FcεRIα, leading to inhibition of FcεRI receptor expression in a cell expressing (i.e., a cell expressing or capable of expressing), an FcεRI receptor.

Accordingly, the invention provides methods and compositions for modulating cell surface expression of an FcεRI receptor in a cell (e.g., contained in a tissue or a subject) which expresses an FcεRI receptor. For ease of discussion, the phrase "cell expressing" is meant to embrace cells already expressing a particular polypeptide, as well as cells capable of such expression. More specifically, the invention is based, in part, on the discovery that expression of a variant of one of the constituent FcεRI receptor chains (e.g., an FcεRIβ chain variant, an FcεRIα chain variant, or an FcεRIγ chain variant) in a cell that expresses a wild type FcεRI receptor, results in the abrogation or decrease of FcεRI receptor cell surface expression. As a result, activity usually associated with such a receptor and mediation of IgE signals are also abolished. The invention, therefore, is useful whenever it is desirable to modulate such activity, for example, in the treatment of conditions mediated by IgE. Thus, the invention also provides methods and related compositions for identifying pharmacological agents useful in the treatment of such conditions.

As used herein, "inhibit expression" refers to inhibiting (i.e., reducing to a detectable extent) replication, transcription, and/or translation of one or more FcεRI receptor constituent polypeptide genes [i.e., the gene encoding the α chain (FcεRIα), the β chain (FcεRIβ), or the γ chain (FcεRIγ)], since inhibition of any of these processes results in the inhibition of expression of an FcεRI receptor constituent polypeptide encoded by its respective gene. The

term also refers to inhibition of post-translational modifications on the FcεRI receptor constituent polypeptide, since inhibition of such modifications will also prevent proper expression (i.e., expression as in a wild type cell) of the encoded polypeptide. The inhibition of gene expression can be directly determined by detecting a decrease in the level of mRNA for the gene, or the level of protein expression of the gene, using any suitable means known to the art, such as nucleic acid hybridization or antibody detection methods, respectively. Inhibition of gene expression can also be determined indirectly by detecting a change in FcεRI receptor activity as a whole (e.g., histamine and/or cytokine release by the cell upon triggering with IgE, etc.), or activity of each of the constituent FcεRI receptor chains (e.g., phosphorylation, glycosylation, etc.).

An "FcεRIβ chain variant" as used herein, refers to a wild type FcεRIβ chain nucleic acid or polypeptide which contains one or more modifications (as described below) in its primary sequence, giving rise to a peptide with functional properties that differ from those of the wild type. In certain instances, for example, the variant will have "dominant negative" peptide properties. In other instances, the variant will have a direct effect on the expression of the FcεRIα and FcεRIγ receptor chains (e.g., at the nucleotide and/or amino acid level - transcriptionally, translationally, post-translationaly), resulting in inhibition of FcεRI receptor expression. A "dominant negative" peptide is an inactive variant of a protein, which, by interacting with the cellular machinery, displaces an active protein (e.g., wild type FcεRIβ chain) from its interaction with the cellular machinery or competes with the active protein, thereby reducing the effect of the active protein. For example, a dominant negative receptor which binds a ligand but does not transmit a signal in response to binding of the ligand can reduce the biological effect of expression of the ligand. Likewise, a dominant negative catalytically-inactive kinase which interacts normally with target proteins but does not phosphorylate the target proteins can reduce phosphorylation of the target proteins in response to a cellular signal. Similarly, a dominant negative transcription factor which binds to a promoter site in the control region of a gene but does not increase gene transcription can reduce the effect of a normal transcription factor by occupying promoter binding sites without increasing transcription. The end result of the expression of a dominant negative polypeptide in a cell is a reduction in function of active proteins. As used herein, therefore, an FcεRIβ chain variant refers to a nucleic acid with a nucleotide sequence as set forth in SEQ ID NO:3, a polypeptide with an amino acid sequence as set forth in SEQ ID NO:4, and structurally related nucleic acids and polypeptides, respectively, which share a common function with

these nucleic acids and polypeptides, namely, the ability to function in a dominant negative fashion and inhibit expression of the FcεRI receptor in screening assays such as those described herein.

By "structurally related," as used herein, refers to nucleic acids and polypeptides that are homologous and/or allelic to the FcεRIβ chain variant. In general homologs and alleles typically will share at least 40% nucleotide identity and/or at least 50% amino acid identity to SEQ ID NO:3 and SEQ ID NO:4, respectively, in some instances will share at least 50% nucleotide identity and/or at least 65% amino acid identity and in still other instances will share at least 60% nucleotide identity and/or at least 75% amino acid identity. The homology can be calculated using various, publicly available software tools developed by NCBI (Bethesda, Maryland). Exemplary tools include the heuristic algorithm of Altschul SF, et al., (*J Mol Biol*, 1990, 215:403-410), also known as BLAST. Pairwise and ClustalW alignments (BLOSUM30 matrix setting) as well as Kyte-Doolittle hydropathic analysis can be obtained using public (EMBL, Heidelberg, Germany) and commercial (e.g., the MacVector sequence analysis software from Oxford Molecular Group/enetics Computer Group, Madison, WI). Watson-Crick complements of the foregoing nucleic acids also are embraced by the invention.

Modifications which create an FcεRIβ chain variant are typically made to the nucleic acid which encodes the FcεRIβ chain polypeptide. Other similar methods for creating and testing variants of a protein according to the invention will be apparent to one of ordinary skill in the art. Thus functionally equivalent variants of FcεRIβT chain polypeptides, i.e., variants of FcεRIβ polypeptides which retain the above-identified function of the FcεRIβT polypeptide, are contemplated by the invention.

One of ordinary skill in the art can screen for a dominant negative variant of a protein, and using standard mutagenesis techniques to create one or more dominant negative FcεRIβ chain variant polypeptides. For example, given the teachings contained herein of FcεRIβ chain variant nucleic acids and polypeptides, one of ordinary skill in the art can modify the FcεRIβ nucleotide (SEQ ID NO:1) and/or peptide sequence (SEQ ID NO:2) by site-specific mutagenesis, scanning mutagenesis, partial gene deletion or truncation, and the like. See, e.g., U.S. Patent No. 5,580,723 and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. The skilled artisan then can test the population of mutagenized polypeptides for their effect in the inhibition of FcεRI receptor expression in a cell expressing (i.e., a cell expressing or capable of

expressing) an FcεRI receptor. These results can be compared to the results obtained in a control experiment, typically an identical experiment performed side-by-side utilizing an FcεRIβ chain variant polypeptide of the invention (e.g., the preferred FcεRIβ chain variant polypeptide, FcεRIβT, having an amino acid sequence as set forth in SEQ ID NO:4). Methods for detecting FcεRI receptor expression inhibition are described elsewhere herein.

In another important aspect, the invention provides a method for inhibiting expression of an FcεRI receptor in a cell of a subject to treat a condition mediated by IgE. The method involves administering to a subject in need of such treatment an FcεRIβ chain variant in an effective amount to inhibit FcεRI receptor expression in a cell of the subject. A "subject" as used herein, is a human, non-human primate, cow, horse, pig, sheep, goat, dog, cat, or rodent. A "cell" of a subject, as used herein, refers to a cell that expresses, or is capable of expressing, an FcεRI receptor, and it includes cells of hematopoietic origin, and more specifically mast cells and/or basophils. A "condition mediated by IgE" as used herein, refers to Type I hypersensitivity responses (also known as immediate hypersensitivity reactions) and IgE-dependent "late phase reactions," characterized by an infiltration of inflammatory cells that appears days after exposure to an allergen (e.g., as in the inflammatory reaction present in the airways of asthmatic patients).

The immune system of humans and animals normally functions to protect its host from infectious organisms or from cancerous transformation by host cells. In many instances however, the immune system manifests a response that itself results in considerable damage to otherwise healthy cells and organs. Such over-reactivity of immune responsiveness is responsible for many serious conditions or diseases including allergies and autoimmune diseases. In order to classify the processes by which the immune system produces cellular damage, immunologists have divided immune responses into four broad classes (Type I, II, III and IV) (Roitt, I. M., et al., Immunology, C. V. Mosby, N.Y., 1985, p. 19.1). "Type I hypersensitivity responses" are also called immediate hypersensitivity reactions and refer to those conditions which produce the symptoms classically associated with "allergies" or the "allergic syndrome" including atopy [e.g., allergic rhinitis (hay fever), allergic asthma, allergic conjunctivitis], urticaria, angioedema, and allergic reactions to insect stings or foods (anaphylaxis). These allergic conditions are characterized by a rapid clinical manifestation of allergic symptoms within minutes after exposure to an antigen (allergen) to which the subject has been previously sensitized.

In order for Type I hypersensitivity to occur, a specialized sequence of events within mast cells and basophils must be triggered by immunoglobulin E (IgE) antibodies that have been manufactured within the body. In this process IgE directed toward an antigen (allergen) must bind to a receptor (FcεRI receptor) on mast cells and basophils which specifically bind to the Fc region of IgE. Mast cells and basophils that have anti-allergen-IgE bound to them are considered to be sensitized or "armed" for subsequent exposure to allergen. Should allergen be introduced into the local environment of the mast cells or basophils, the cells are automatically stimulated or "triggered" to release histamine and other vasoactive chemicals (e.g., lipid mediators such as leukotrienes B_4 and C_4, prostaglandin D_2, platelet-activating factor, cytokines such as IL-4, IL-5, IL-6, TNF-α, etc.) which produce the familiar "allergic symptoms" characteristic of allergic conditions.

The hypersensitivity states characterized by types II, III and IV hypersensitivity are very distinct from type I hypersensitivity. For example, allergic inflammation in type I hypersensitivity (allergy) begins within minutes after allergen exposure. By contrast, other hypersensitivity states exhibit inflammation only after hours to days following reexposure to the sensitizing agent. Additionally, in type I hypersensitivity, the sensitizing agent (allergen) is not a part or component of the host body but a substance found outside of the host body that is later introduced into the body by exposure to the environment. Types II, III and IV hypersensitivity, by contrast, may have immune responses directed towards antigens located on cells and molecules that are normal constituents of the body. Such immune responses toward normal constituents of the body are termed "autoimmune diseases" and constitute a medically class of diseases distinct from conditions mediated by IgE (e.g., allergies). A further distinction is the degree to which cell killing occurs. In type I hypersensitivity, the IgE mediated triggering reaction which causes the release of vasoactive allergic mediators does not result in the death of the releasing mast cell or basophil. Instead, the "trigger" reaction is the result of an active secretory process that may recur after a length of time. Similarly, the effect of the vasoactive allergic mediators on surrounding cells is regulatory, not cytotoxic. Allergic mediators serve to increase the permeability of small blood vessels and activate a variety of vasoregulatory and immunoregulatory processes that do not normally result in cell death. Types II, III and IV hypersensitivity, by contrast, have as a principal function cell killing reactions which normally lead to the destruction of infectious agents or cancer cells.

In a further aspect, the invention involves a method of screening for FcεRI receptor expression modulating agents. The method involves (a) contacting a putative FcεRI receptor expression modulating agent with a cell expressing (i.e., a cell expressing or capable of expressing) an FcεRI receptor, (b) measuring FcεRI receptor expression by the cell, and (c) determining whether FcεRI receptor expression by the cell is altered compared to FcεRI receptor expression by a control cell, wherein the control cell is contacted with an FcεRIβ chain variant. Additional controls may also include measuring FcεRI receptor expression by a cell in the absence of a putative FcεRI receptor expression modulating agent. As mentioned, *supra*, "measuring FcεRI receptor expression by the cell" is accomplished using a number of different methods, most of which are well known by a person of ordinary skill in the art. For example, a direct way would be to measure mRNA expression for one of the constituent FcεRI receptor chains using Northern blotting, RT-PCR, etc. The human nucleic acid sequences encoding each of the three constituent FcεRI receptor chains are known in the art and are publicly available through NCBI's GenBank databases (Accession nos.: X06948 for FcεRIα -SEQ ID NO:7; M89796 for FcεRIβ-SEQ ID NO:1; and L03533 for FcεRIγ -SEQ ID NO:8). Another direct way to measure FcεRI receptor expression by the cell is to use antibodies specific for one of the constituent FcεRI receptor chains and a number of immunocyto- and immunohisto- chemical protocols. Antibodies specific for each of the constituent FcεRI receptor chains are commercially available and can be obtained, for example, through Upstate Biotechnology, Lake Placid, NY [rabbit polyclonal anti-human FcεRIα peptide (997) cat. 06-725; mouse monoclonal anti-human FcεRIα chain (clone 3G6) cat. 05-491; rabbit polyclonal anti-human FcεRIβ peptide cat. 06-726; rabbit polyclonal anti-human FcεRIγ peptide (934) cat. 06-727]. In a preferred embodiment, expression of more than one chain is measured at the same time. For example, expression of the FcεRIα chain is measured at the same time as FcεRIβ chain expression. Another indirect way to measure FcεRI receptor expression by the cell is to measure FcεRI receptor activity. For example, FcεRI receptor activity can be measured by the downstream effects of IgE binding to the receptor. Such downstream effects include secretion of chemical compounds by the cell, *supra*, in response to antigen-IgE binding to the receptor.

In one embodiment, the FcεRIβ chain variant is an endogenous nucleic acid molecule of the cell. By "endogenous" it is meant that it is naturally present in the cell genome. In another embodiment the FcεRIβ chain variant is a heterologous nucleic acid molecule of the

cell. As used herein, "heterologous" or foreign nucleic acid molecule (DNA and RNA) are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the genome in which it is present or which is found in a location or locations in the genome that differ from that in which it occurs in nature. Preferably, it is DNA or RNA that is not endogenous to the cell and has been artificially introduced into the cell. Examples of heterologous DNA include, but are not limited to, DNA that encodes an FcεRIβ chain variant polypeptide according to the invention, and DNA that encodes RNA or proteins that mediate or alter expression of endogenous DNA (for example, that of wild type FcεRIβ chain) by affecting transcription, translation, or other regulatable biochemical processes. The cell that expresses the heterologous DNA, such as DNA encoding an FcεRIβ chain variant polypeptide, may contain DNA encoding the same or different FcεRIβ chain variant polypeptide.

Generally, the screening methods of the invention involve assaying for compounds which interfere with FcεRI receptor expression activity, and can be detected by any of the above-identified methods (e.g., downstream effects after the FcεRI receptor binds its natural ligand, IgE). Such methods are adaptable to automated, high throughput screening of compounds. The target therapeutic indications for agents detected by the screening methods are limited only in that the target cellular function be subject to modulation by alteration of the formation of a complex comprising an FcεRI receptor, and its natural ligand, IgE. Target indications can include cellular processes mediated by the FcεRI receptor following its binding to IgE (e.g., histamine or cytokine release, etc.).

In addition to the FcεRI receptor, a screening assay mixture includes a binding partner for the receptor, e.g., a naturally occurring ligand (i.e., IgE) that is capable of binding to the FcεRI receptor or, alternatively, is comprised of an analog which mimics the FcεRI receptor binding properties of the naturally occurring ligand for purposes of the assay. The screening assay mixture also comprises a candidate agent (e.g., an agent that modulates expression of the FcεRI receptor, and most preferably inhibits expression of the FcεRI receptor).

Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. An essential control according to the invention is the comparison of the effects of the inhibitory agent on FcεRI receptor expression with the FcεRI receptor expression in a cell expressing (i.e., a cell expressing or

capable of expressing) an FcεRIβ chain variant of the invention. Candidate agents encompass numerous chemical classes, although typically they are organic compounds. Preferably, the candidate agents are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500, preferably less than about 1000 and, more preferably, less than about 500. Candidate agents comprise functional chemical groups necessary for structural interactions with polypeptides and/or nucleic acids, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups and more preferably at least three of the functional chemical groups. The candidate agents can comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted with one or more of the above-identified functional groups. Candidate agents also can be biomolecules such as peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the agent is a nucleic acid, the agent typically is a DNA or RNA molecule, although modified nucleic acids as defined herein are also contemplated.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides, synthetic organic combinatorial libraries, phage display libraries of random peptides, and the like. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds can be readily be modified through conventional chemical, physical, and biochemical means. Further, known pharmacological agents may be subjected to directed or random chemical modifications such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs of the agents.

A variety of other reagents also can be included in the mixture. These include reagents such as salts, buffers, neutral proteins (e.g., albumin), detergents, etc. which may be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent may also reduce non-specific or background interactions of the reaction components. Other reagents that improve the efficiency of the assay such as protease inhibitors, nuclease inhibitors, antimicrobial agents, and the like may also be used.

The mixture of the foregoing assay materials is incubated under conditions whereby, but for the presence of the candidate putative inhibitor agent, the FcεRI receptor specifically binds its natural binding target, a portion thereof or analog thereof. The order of addition of components, incubation temperature, time of incubation, and other parameters of the assay may be readily determined. Such experimentation merely involves optimization of the assay parameters, not the fundamental composition of the assay. Incubation temperatures typically are between 4°C and 40°C. Incubation times preferably are minimized to facilitate rapid, high throughput screening, and typically are between 0.1 and 10 hours.

After incubation, the presence or absence of specific binding between the FcεRI receptor and one or more binding targets is detected by any convenient method available to the user, *supra*.

The therapeutics of the invention embrace isolated nucleic acids and polypeptides. The term "isolated", as used herein in reference to a nucleic acid molecule, means a nucleic acid sequence: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) synthesized by, for example, chemical synthesis; (iii) recombinantly produced by cloning; or (iv) purified, as by cleavage and gel separation. The term "isolated", as used herein in reference to a polypeptide (protein), means a polypeptide encoded by an isolated nucleic acid sequence, as well as polypeptides synthesized by, for example, chemical synthetic methods, and polypeptides separated from biological materials, and then purified using conventional protein analytical procedures.

The therapeutics of the invention are in effective amounts. The "effective amount" will depend upon the mode of administration, the particular condition being treated and the desired outcome. It will also depend upon, the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result. In some cases, for example, this is a decrease in a subject's immune hypersensitivity to allergens as evidenced by a decrease in circulating histamines, cytokines, etc. (i.e., inhibition of allergen effects).

Generally, doses of active compounds of the present invention would be from about 0.01 mg/kg per day to 1000 mg/kg per day. It is expected that doses ranging from 50-500 mg/kg will be suitable. A variety of administration routes are available. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active

compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, intradermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. They could, however, be preferred in emergency situations. Oral administration will be preferred for prophylactic treatment because of the convenience to the patient as well as the dosing schedule. When peptides are used therapeutically, in certain embodiments a desirable route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing peptides are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the peptides, for example, the paratope binding capacity of a peptide (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing antibody or peptide aerosols without resort to undue experimentation.

Preferred methods for administering FcεRIβ variants or agents that induce FcεRIβ variant expression of the invention also include spliceosome-mediated RNA trans-splicing as described by Puttaraju, M., et al (53) and using agents commercially available from Intronn LLC (Durham, NC), and ribozymes using methods well known in the art and reviewed by Woolf, TM (58).

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active agent. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Lower doses will result from other forms of administration, such as

intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

The FcεRIβ chain variant polypeptides or functionally equivalent fragments thereof may be combined, optionally, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. Such preparations may routinely contain salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

FcεRIβ chain variant polypeptides, or functionally equivalent fragments thereof, preferably are produced recombinantly, although such polypeptides may be isolated from biological extracts. Recombinantly produced FcεRIβ chain variant polypeptides include chimeric proteins comprising a fusion of a FcεRIβ chain variant peptide with another polypeptide, e.g., a polypeptide capable of providing or enhancing protein-protein binding, sequence specific nucleic acid binding (such as GAL4), enhancing stability of FcεRIβ chain variant polypeptide under assay conditions, or providing a detectable moiety, such as green fluorescent protein. A polypeptide fused to a FcεRIβ chain variant polypeptide or fragment

may also provide means of readily detecting the fusion protein, e.g., by immunological recognition or by fluorescent labeling.

Various techniques may be employed for introducing FcεRIβ chain variant encoding nucleic acids of the invention into cells, depending on whether the nucleic acids are introduced *in vitro* or *in vivo* in a host. Such techniques include transfection of nucleic acid-$CaPO_4$ precipitates, transfection of nucleic acids associated with DEAE, transfection with a retrovirus including the nucleic acid of interest, liposome mediated transfection, and the like. For certain uses, it is preferred to target the nucleic acid to particular cells. In such instances, a vehicle used for delivering a nucleic acid of the invention into a cell (e.g., a retrovirus, or other virus; a liposome) can have a targeting molecule attached thereto. For example, a molecule such as an antibody specific for a surface membrane protein on the target cell or a ligand for a receptor on the target cell can be bound to or incorporated within the nucleic acid delivery vehicle. For example, where liposomes are employed to deliver the nucleic acids of the invention, proteins which bind to a surface membrane protein associated with endocytosis may be incorporated into the liposome formulation for targeting and/or to facilitate uptake. Such proteins include capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half life, and the like. Polymeric delivery systems also have been used successfully to deliver nucleic acids into cells, as is known by those skilled in the art. Such systems even permit oral delivery of nucleic acids.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the anti-inflammatory agent, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the anti-inflammatory agent is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,667,014, 4,748,034 and 5,239,660

and (b) difusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,832,253, and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions. Long-term release, are used herein, means that the implant is constructed and arranged to delivery therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

In other aspects, the agents of the invention are "co-administered," which means administered substantially simultaneously, with another anti-allergic agent other than an FcεRIβ chain variant molecule. By substantially simultaneously, it is meant that an FcεRIβ chain variant related molecule of the invention is administered to a subject, as an admixture in a single composition, or sequentially, close enough in time with the administration of the other anti-allergic agent, whereby the two compounds may exert an additive or even synergistic effect, e.g. decreasing or completely eliminating a Type I hypersensitivity response.

The invention also embraces determining whether a subject has a condition mediated by IgE or a predisposition thereto. The method involves determining whether an FcεRIβ chain variant according to the invention is present in a subject, and/or whether this variant is expressed at different levels in the subject when compared to a control. In some embodiments, the method involves determining whether the FcεRIβ chain variant is present and/or expressed at lower levels (amounts) in the subject in comparison to a control. By "control" as used herein, refers to FcεRIβ chain variant expression in a control subject. A "control subject" is an apparently healthy subject with no symptoms indicative of a condition mediated by IgE, or predisposition to a condition mediated by IgE (e.g., no family history of a condition mediated by IgE). In a preferred embodiment, the FcεRIβ chain variant is a polypeptide designated herein as FcεRIβT having an amino acid sequence as set forth in SEQ ID NO:4. Variant polypeptide FcεRIβT arises from an FcεRIβ chain mRNA (having a cDNA as depicted in SEQ ID NO:3), in which intron 5 (402 nucleotides in length) of the genomic FcεRIβ chain gene, is not spliced out and forms part of the mRNA. The resulting translated variant polypeptide, FcεRIβT, is shorter than the wild type FcεRIβ polypeptide because of an *in frame* early termination signal in the the fifth intron and, as a result, is missing the C-terminus of the wild type protein (see Figure 1).

In determining the presence and/or amount of an FcεRIβ chain variant of the invention, a suitable tissue sample is obtained from the subject suspected of having a condition mediated by IgE or a predisposition thereto. Lymph or blood are the preferred tissues where samples can be obtained, but other tissues that contain cells of hematopoietic origin (such as mast cells and basophils) can also be used according to the invention. A preferred method for determining the presence and/or levels of FcεRIβ chain variant expression according to this aspect of the invention is quantitative RT-PCR (54-57). Given the teachings of the present invention and the public availability of the wild type FcεRIβ genomic sequence, the skilled artisan can easily select a pair of primers that spans any of the introns of the FcεRIβ genomic sequence. In certain embodiments, primers that span intron 5 (e.g., from exons 2 and 7) to perform the detection and/or quantitation of the variant trancript are selected. Examples of such primers include those whose sequences are set forth in SEQ ID NOs 5 and 6. The assay is performed in parallel with or in reference to a control assay in which samples obtained from control subjects, preferably of like tissue, are used to establish a control amount of the variant. The wild type transcript optionally serves as an internal control for the reaction and/or quantitation. In some embodiments, a lower level (amount) of the FcεRIβ chain variant transcript relative to an FcεRIβ chain variant transcript of a control subject, is indicative of the subject having a condition mediated by IgE or a predisposition thereto.

The invention will be more fully understood by reference to the following examples. These examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention.

Examples

Experimental procedures

Cell Culture

NIH3T3 $\alpha\beta\gamma_2$ transfectants were maintained in DMEM (Biofluids, Rockville, MD) with 10% calf serum (Biofluids, Rockville, MD) and 300 μg/ml of hygromycin (Calbiochem, La Jolla, CA) and 500 μg/ml of neomycin (Gibco/BRL, Baltimore, MD). All the other NIH-3T3 transfected cell lines were maintained in the same medium without neomycin. U937 transfected cell lines were maintained in RPMI-1640 (Biofluids) with 20% fetal bovine serum (Biofluids) and 1 mg/ml of neomycin. Mouse Bone Marrow-derived Mast Cells (BMMC) were obtained as previously described (42, 43). Human Cord Blood Mast Cells were

generated as previously described (44). In brief, mononucleated cells were isolated from human umbilical cord blood by centrifugation over Histopaque-1077 (Sigma), and then were cultured in Iscove's modified Dulbecco's medium supplemented with 10 % FBS, 100 ng/ml recombinant human stem cell factor (Biosource), 10 ng/ml recombinant human IL-6 (Endogen) and 1 mM prostaglandin E2 (Cayman Chemical, Ann Arbor, MI). The entire culture medium was changed 3 times a week during the first 2 weeks, then half of it was replaced weekly, and the cells were maintained in culture for at least 10 weeks. Human basophil-enriched leukocyte suspensions were prepared over a single-step Percoll gradient according to an etablished protocol (45).

Expression Vectors

The human α subunit and mutated forms of rat β cDNA (βYM, tyrosines at position 218, 224, and 228 with the βITAM were changed to phenylalanines) of FcεRI were subcloned into pCDL-SRα 296. The rat α and γ subunits of FcεRI were subcloned into pSHSX, a pCDL-SRα 296 derivative containing a hygromycin resistance cassette. Human β and γ subunit, rat β subunit cDNAs and the mutated form of rat γ cDNA (γYM, tyrosines at position 65 and 76 within the γ ITAM were changed to phenylalanines) were subcloned into pBJ1neo, a neomycin derivative of pCDL-SRα 296 (15). The trunctated forms of rat β (βNT, the first 57 amino acids of NH_2-terminal cytoplasmic domain were deleted; βCT, the last 38 amino acids of COOH-terminal cytoplasmic domain were deleted) have been described (40). βNT and βCT were subcloned into pCDL-SRα 296. To construct the181L-183L and 237G β variants, the WT β cDNA subcloned in the eukaryotic expression vector pBJ1neo was used as a template for site-directed mutagenesis with the Quick-change kit (Stratagene) using two different oligonucleotides containing the sequences from the 181L-183L and 237G variants. Confirmation of the mutations was obtained by DNA sequencing. For the spice variant of human FcεRIβ (see RT PCR paragraph below). The βWT and splice variant cDNAs were FLAG-tagged at the N terminus using the FLAG epitope (Kodak, New Haven, CO). The flagged cDNAs were subcloned in pBJ1neo.

Antibodies

The monoclonal anti-phosphotyrosine antibody (4G10) was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). PE-streptavidin was from PharMingen (San Diego, CA). The monoclonal anti-DNP IgE used in culture supernatant form, rabbit anti-mouse IgE, anti-rat FcεRIβN tail monoclonal antibody (JRK), rabbit anti-FcRγ subunit antibody and monoclonal anti-human-FcεRIα subunit antibody (15-1), were prepared as described (15).

Rabbit anti-porcine *syk*, and anti-murine *lyn* were described previously (14). The anti-human *syk* antibody 996 was raised against a synthetic peptide representing amino acids 150-159 of human syk sequence (15). Monoclonal anti-rat FcεRIβC tail antibody (NB) was from Dr. D Holowka, and chimeric anti-Nip human IgE were from Dr. Z. Eshhar. The monoclonal FLAG antibody covalently attached to agarose beads was from Sigma (Saint Louis, MO).

Recombinant Vaccinia Virus Construction

Recombinant vaccinia virus expressing murine *Lyn* A kinase, porcine *Syk* kinase, and virus with the empty pSC-65 vaccinia recombinant plasmid were constructed as described (14).

Construction of NIH-3T3 and U937 sublines

The generation of $\alpha\beta\gamma_2$ cells has been previously described (14). Construction of other NIH-3T3 sublines expressing FceRI variants was similar. In brief, cells were co-electroporated (270V, 960μF) with appropriate constructs. Resistant clones were selected, and screened for surface FcεRI expression by flow cytometry with FITC-IgE. U937 cells were co-transfected by electroporation (260V, 960μF) with human α, β and γ constructs or with α and γ constructs only. Cells were grown under 1 mg/ml G418 selection, and resistant clones were selected for FcεRIα surface expression by binding with biotinylated human IgE and PE-labeled streptavidin. Two clones of each type (αβγ-2 and αβγ-8, αγ-1 and αγ-10) were selected for analysis. Transient transfection KU812 cells (5×10^6) were co-transfected by electroporation (300V, 960 μF) with 10 μg of βT expression vector and 1μg of green fluorescent protein (GFP; pGreen Lantern, Gibco). Human CD81 subcloned in PBJ1neo was used as a control vector (Fleming 1997). At different times after the transfection, FceRI expression was analysed on GFP positive gated cells.

Infection with Recombinant Vaccinia Viruses

Adherent NIH 3T3 cells in one $150cm^2$ culture flask were infected with the appropriate virus at 5 pfu/cell in 5 ml DMEM with 2.5% calf serum for 30 min at 4°C with gentle rocking, then for 30 min at 37°C. Control virus bearing the pSC65 vaccinia recombinant vector was added to single recombinant virus infection in order to allow valid comparisons with recombinant virus coinfections.

Cell Activation with Antigen and Lysis

Infected NIH-3T3 sublines ($3\text{-}5 \times 10^6$ cells/sample) or U937 sublines (3×10^7 cells/sample) were harvested in 1 ml medium. Cells were stimulated with 200 ng/ml DNP24-40-HSA (Sigma, St Louis, MO) in 1 ml for 4 min at room temperature with rocking. Towards

the end of stimulation, cells were pelleted briefly in a microcentrifuge, the medium was aspirated and the cell pellet was resuspended in ice cold lysis buffer (0.5% TritonX-100, 150 mM NaCl, 200 mM boric acid [pH 8.0], 5 mM EDTA, 5 mM sodium floride, 1 mM sodium vanadate, 10 μg/ml pepstatin, 5 μg/ml leupeptin, 10 μg/ml aprotinin) at a ratio of 3 x 10^7 cells/ml of lysis buffer, and kept on ice for 10-15 min. Lysates were spun for 10 min at 14,000 rpm to remove cell nuclei prior to immunoprecipitation or SDS-PAGE. In experiments involving $\alpha\beta T\gamma_2$ receptor, cells were lysed in 10 mM CHAPS instead of 0.5 % TritonX-100.

Immunoprecipitations, In Vitro Kinase Assays, Western Blotting

Immunoprecipitations were performed as previously described (14), except for the cells lysed in 10 mM CHAPS. In this case immunoprecipitates were washed with lysis buffer containing 2 mM CHAPS. *In vitro* kinase assays were performed as described (15), and analyzed by SDS-PAGE and autoradiography. Where indicated, immunoprecipitates were treated with endo-β-N-acetylglucosamidase (Endo H) (New England Biolabs) as previously described (47). After immunoprecipitation with appropriate antibodies, samples were resolved on SDS polyacrylamide gel, transferred to PVDF membrane, and blotted with the antibodies indicated. Immunoreactive proteins were visualized by using alkaline phosphatase-coupled second-step reagents and enhanced chemifluorescence (ECF, Amersham). Fluorescence was quantified using a Storm scanner (Molecular Dynamics). Where appropriate, cells were treated with proteasome inhibitors (ALLN 250 μM) (Calbiochem) or vehicle for 2 hours at 37°C before lysis.

Measurement of Calcium Moblization

U937 transfectant cells were washed and incubated with 2 μM Fura-2 acetoxymethyl ester (Moleular Probes, Portland, OR) and 0.08% Pluronic F-127 (Molecular Probes) in calcium buffer (135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 10 mM HEPES, pH 7.4, 0.1% BSA and 2.5 mM Probenecid [Sigma, St. Louis, MO]) for 1 hr with gentle rocking at room temperature. Cells were washed once in the same buffer, and transferred into the cuvette of a Deltascan spectrofluorometer (Photon Technology International Inc., South Brunswick, NJ). Fura-2 loaded cells (5 x 10^6 cells in 2 ml calcium buffer) were exposed to 5 μg/ml of biotinylated human IgE for 10 min before the addition of 25 μg/ml streptavidin at at room temperature. Calcium moblization was monitored and quantified as previously described (15).

Targeting of FcRβ Gene

The mouse FcRβ gene was isolated from a 129 λ-DASH library (49) (a gift from Dr. P. Love, NIH, Bethesda, MD). Targeted disruption of the gene was achieved by replacement of exons IV-VI (corresponding to bp 344-577 of the mouse FcRβ cDNA sequence -SEQ ID NO:9) (GenBank Acc. No. AB033617, and 50) with a Neo cassette. Briefly, a genomic 4 kb fragment encompassing exons I-IV (bp 38-343 of the mouse FcRβ cDNA sequence) and a genomic 1.4 kb fragment encompassing exons VI-VII were amplified by polymerase chain reaction using appropriate primers and cloned respectively into the XhoI and XbaI of pJNS2 vector (42). The construct was linearized by NotI and electroporated into D3 or E14 TG2a ES cells. ES cells were grown and selected as previously described (48). Genomic DNA was extracted from clonal ES cells, digested with DraI and hybridized by Southern blot to a 440 bp fragment located 16 bp downstream of exon VII. The hybridizing bands were 3.4 kb for the WT and 2.8kb for the disrupted allele. Two out of 109 G418 resistant-ganciclovir sensitive clones were found positive for homologous recombination.

Animals

Chimera, heterozygous (+/-) and homozygous (-/-) animals for the disrupted FcRβ allele were generated as previously described (48); FcRβ -/- animals were on a Balb/c F2 background. FceRIα -/-animals have been previously described (42), and were on a Balb/c F6 background. FcRβ -/- animals were compared to age-matched FcεRIα -/- for all relevant experiments. FcRβ -/- were also crossed with the previously described transgenic mice for the human FcεRIα gene (hFcεRIαTg) (43). Progeny of the second generation thus contained hFcεRIαTg/FcRβ-/- or hFcεRIαTg/FcRβ +/- animals. Littermates were used in all appropriate experiments. Human hFcεRIαTg mice used for these crosses were in a Balb/c WT background, so that hFcεRIαTg/FcRβ +/- animals were expressing both human and murine FcεRI on their mast cells. Animals used for the study of hFcεRI cellular distribution were on a FcεRIα -/- background and were thus expressing only hFcεRI.

Degranulation

Cellular degranulation was measured by the release of β-hexoseaminidase as described before with minor modifications (51). Cells were incubated with 50 ng/ml/10^6 cells humanIgE over night or with various doses of anti- FcεRIα (15-1) for 2 hours, washed twice and stimulated with NIP-BSA or with 10 μg/ml goat anti-mouse IgG $F(ab')_2$ for 20 min.

Anaphylaxis

Anaphylactic reactions were induced and measured as described in Dombrowicz et al., 1997 (52), except that human IgE-induced systemic anaphylaxis was achieved by 3 *iv* injections of 50 μg anti-NIP hIgE in 200 μl PBS at 12h intervals followed 4h later by an *iv* challenge with 1mg NIP-BSA in 200 μl PBS.

RT-PCR

Total RNA was isolated with RNAzol B (Tel-Test, Inc., Friendswood, TX) following manufacturer instructions. Aliquots of 1 μg of total RNA were converted to cDNA by using poly(dT)18 primers and transcribed with 100 units of Moloney murine leukemia virus reverse transcriptase (Clontech, Palo Altp, CA) at 42°C for 60 min. For PCR, aliquots of DNA equivalent to 0.1 μg total RNA were used in each reaction (50 μl) containing 50 pmol of each primer, 200 μM of each deoxynucleoside trisphosphate, and 1.25 units of Taq polymerase (Fisher Scientific, Pittsburg, PA). βWT and its splice variant were amplified using a sense primer located in exon 2 (5'-GTGCCTGCATTTGAAGTCTTG-3', SEQ ID NO:5) and antisense primer located in exon 7 (5'-TGGATCCTTGGCTGTGAATC-3', SEQ ID NO:6). The PCR reactions were performed under the following conditions : 1min at 94°C, followed by 22 to30 cycles of 45 sec at 94°C, 45 sec at 60°C, 1 min at 72°C. PCR products were visualised on 1.2 % agarose gels stained with ethidium bromide.

Cloning of PCR Products

PCR products were isolated after agarose gel separation, purified with QIAquick PCR Purification kit (QIAGEN, Valencia, CA) and cloned into a pCRII vector using TA cloning (Invitrogen).

RNase Protection Assay (RPA)

Total RNA from untransfected or transfected U937 cells was prepared with RNAzol B (Tel-Test, Inc., Friendswood, TX). Poly A RNA from CBMC was purified using the FastTrack 2.0 kit (Invitrogen, Carlsbad, CA). Antisense radioactive RNA probes were generated by in vitro transcription using the Maxiscript kit (Ambion, Austin, TX). The β probe was designed to overlap the end of exon 5 and the beginning of intron 5. Probes were also designed in the α and γ cDNAs and used as controls. RPA was performed using the RPAIII kit (Ambion, Austin, TX). Ten μg total RNA from U937 cells and the mRNA obtained from 30 x 10^6 CBMC were incubated with 10^5 cpm freshly prepared probe, and probe was then digested in the presence of 0.375 units RNAse A and 15 units RNAse T1 or buffer. Protected probe fragments were separated by denaturing polyacrylamide gel electrophoresis. Gels were exposed to fluorescent screens that were read on a Storm 840

scanner (Molecular Dynamics, CA), and analyzed using the ImageQuant software (Molecular Dynamics, CA).

Pulse-Chase Analysis

45 x 10^6 actively growing cells were washed twice in cysteine and methionine-free RPMI medium supplemented with 1 mM L-glutamine and 10 mM Hepes. Cells were then incubated in the same medium containing 10% dialyzed FBS for 20 min at 37°C. This medium was then removed and the cells at 5 x 10^6/ml were pulsed with 0.2 mCi/ml TRAN 35S-LABEL (ICN) for 10 min at 37°C. An aliquot of 15 x 10^6 cells for time 0 was collected, and the remaining cells were washed and resuspended in complete medium supplemented with cysteine and methionine for the chase times indicated. At each time point, 15 x 10^6 cells were pelleted by centrifugation, washed twice with ice-cold PBS and resuspended in 0.5 ml of ice-cold lysis buffer (0.5 % Triton X-100, 300 mM NaCl, 50 mM Tris, pH 7.5) in the presence of protease inhibitors). Samples were then precleared and immunoprecipitated with anti-FLAG antibody as above. Proteins were separated on SDS 14% polyacrylamide gels, and the dried gels were exposed to a Molecular Dynamic phosphor Image screen for 15 h. Radiolabeled proteins detected by PhosphorImager were quantitated with Image-Quant software (Molecular Dynamics).

Example 1: *FcεRIβ is a signal amplifier*

The FcεRIβ molecule is a unique subunit among human antigen receptors. It has a topology where both tails are cytoplasmic, and contains an atypical ITAM motif with an extra tyrosine and decreased spacing relative to the consensus.

We used a fibroblast based reconstitution system to investigate β function. NIH3T3 stably transfected with human FcεRI αβγ cDNAs are infected with vaccinia viruses separately encoding the Lyn and Syk tyrosine kinases. Surface α levels were assessed by flow cytometry and then antigen stimulation of equivalent numbers of FcεRI between clones was performed. When $\alpha\beta\gamma_2$ receptors are stimulated, there is substantially enhanced Syk tyrosine phosphorylation, Syk kinase activity, and FcεRIγ tyrosine phosphorylation relative to that achieved by stimulation of $\alpha\gamma_2$ receptors. These data were subsequently reproduced precisely in a hematopoietic cell line system, the U937. The latter cell line also enabled us to perform a functional assay to assess whether the biochemical amplification observed translated to a significant increase in a functional response to antigen stimulation.

We examined FcεRI induced calcium mobilization in U937 transfected with either $\alpha\gamma_2$ or $\alpha\beta\gamma_2$ receptors. Calcium mobilization by FcεRI is biphasic, an early phase of IP_3-

dependent calcium release from intracellular stores is followed by a sustained calcium influx. We also observed that in two $\alpha\beta\gamma_2$ expressing U937 clones, relative to two $\alpha\gamma_2$ clones, there is a remarkable increase in the magnitude of FcεRI induced calcium signals. This translates the amplification of early signaling events to an enhancement in a functional response. Calcium signals contribute to allergic mediator secretion and controls induction of several cytokine genes (e.g. IL-4, IL-6) in mast cells. Therefore, the data suggested that the presence or absence of β profoundly affected the outcome of FcεRI signaling at the whole animal level.

We generated a novel humanized FcεRIα transgenic mouse to assess the contribution of FcεRIβ to *in vivo* responses. In bone marrow derived mast cells (BMMC) prepared from $\alpha\beta\gamma_2$ or $\alpha\gamma_2$ animals, we found that the biochemical evidence for an amplifier function of β (e.g., enhanced FcεRIγ phosphorylation), exactly paralleled that seen in our *in vitro* system. We also found a similar enhancement of calcium mobilization. Most importantly, we were able to assess *in vivo* functional responses in the absence and presence of β. We observed that secretion of allergic mediators in response to antigen stimulation of BMMC is significantly enhanced in $\alpha\beta\gamma_2$ mice relative to $\alpha\gamma_2$, an effect also observed for IL-6 production. Finally, even systemic anaphylactic responses were amplified by β.

Example 2: *FcεRIβ is an amplifier of receptor surface expression*

Stable cell lines expressing FcεRI isoforms are important tools in our analyses of FcεRI biology. We generated such lines in both fibroblast and hematopoietic cell backgrounds (NIH 3T3 and U937), and analyzed surface FcεRI expression by flow cytometry. There is, in all cases, a statistically significant difference between surface expression of $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ receptors. The presence of β correlates with increased expression of receptor at the cell surface in both NIH3T3 and U937 cells. Moreover, these results were subsequently reproduced in transient expression systems.

These data led us to the intriguing possibility that the presence of β may enhance FcεRI surface levels. We tested the idea that β could inducibly alter FcεRI expression levels. FcεRIβ was transiently transfected into KU812 which had been stably transfected with $\alpha\gamma_2$ receptors. KU812 are a transformed mast cell line with no endogenous expression of FcεRI at their surface. We stained the cells for surface α levels and analyzed the population over a 48h time course. We observed that from 15h after transfection onward, KU812 transfected with FcεRIβ have significantly enhanced FcεRI surface expression relative to controls. Thus, introduction of FcεRIβ induces expression of FcεRI complexes at the cell surface.

Without wanting to be bound by a particular hypothesis, we formulated two hypotheses which, while not mutually exclusive, could explain the apparent ability of β to enhance FcεRI surface expression. First, β could be promoting the trafficking of FcεRI subunits from their sites of synthesis in the ER to the plasma membrane. Second, β may affect the stability of surface and nascent complexes, decreasing their turnover and causing a cumulative increase in surface receptor numbers. We then addressed whether either or both of these mechanisms were likely to operate.

FcεRI complexes undergo the following maturation process: 1) In the ER, subunits associate non-covalently and α is core-glycosylated, and thus sensitive to the action of Endoglycosidase H (Endo H). 2) Trafficking from ER to Golgi follows; in the Golgi, terminal glycosylation occurs which places complex sugars (Endo H resistant) on the α chain. We can use SDS-PAGE to look at trafficking of nascent $\alpha\beta\gamma_2/\alpha\gamma_2$ complexes, differentiating between stages on the basis of molecular weight and EndoH sensitivity.

We compared trafficking of FcεRIα in the absence and presence of FcεRIβ. We used stable cell lines expressing either $\alpha\beta\gamma_2$ or $\alpha\gamma_2$, and immunoprecipitated α chains from lysates treated either with vehicle or Endo H. Samples were resolved by SDS-PAGE and Western analysis was performed using polyclonal anti-α. Three α species are apparently visible. Unglycosylated (early ER) α appears as a single, 30 kDa band; immature α, which is glycosylated, runs at 48 kDa, and is sensitive to Endo H. Endo H treatment leads to a reduction in intensity of this 48 kDa species and a concomitant increase in levels of unglycosylated α. Terminally glycosylated α runs as a diffuse species at around 66 kDa. In contrast with the 48 kDa species, mature, complex-sugar glycosylated, α is resistant to Endo H. Clear differences in α content are observed between $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ cell lines. In the presence of β chains there is less of the Endo H sensitive (immature) α species and an increased amount of mature α chains. Densitometry was performed to quantitate these differences and a ratio of mature to immature α chains was calculated. Thus, the presence of β affects the intracellular trafficking of α chains, apparently accelerating their progress towards a mature form.

Example 3: *A novel splice variant of β, βT, is an intrinsic downregulator of FcεRI surface expression*

When RT-PCR was performed with the pair of β chain specific primers whose sequences are set forth in SEQ ID NOs 5 and 6, on human cord blood derived mast cells or

peripheral blood basophils, a second β transcript is observed, at 1.1 kb, compared with the 0.7 kb expected transcript. The additional transcript is present in all the patient samples analyzed. We hypothesized that this additional mRNA species could correspond to an alternate splice form of β.

The additional PCR product was purified, subcloned and placed in a mammalian expression vector. Sequencing revealed that the variant form of β was indeed a product of alternate splicing. The domain structure of the human FcεRIβ gene is shown in Figure 1. In the alternate splice form of β, intronic sequence from the fifth intron contributes a novel 16 aa sequence that replaces the normal β carboxyl terminus and terminates with a stop codon from the intron.

The splice variant of β would produce a truncated protein, βT, lacking the C-terminal transmembrane and cytoplasmic domains. Since the new 16 aa sequence has some hydrophobic character, it is possible that there is some interaction with the membrane to form an imperfect transmembrane domain. Alternatively, βT may be topologically distinct from wild-type (βWT), with the C- and N-termini on opposite sides of the membrane.

In order for the splice variant to have any important biological function, it is a prerequisite that both the transcript and truncated protein are actually produced. To test whether this was the case, we first performed RNAse protection assays (RPA) to test that βT was a transcript that could be detected in normal cell and was not a PCR artifact. RPA can detect as little as 5 fg RNA. We used an antisense probe covering the 3' end of β exon 5 and the 5' end of β intron 5. We performed the RNase Protection Assay in U937 cells (untransfected, or transfected with either $\alpha\beta\gamma_2$ or $\alpha\beta T\gamma_2$), or primary human cord blood derived mast cells.

We next assessed whether βT was translated into a protein. In Western analysis of CBMC lysates, the βT signal was present, but extremely hard to visualize. Transfection of βT into U937 caused the appearance of a band that co-migrated with the low molecular weight form of β seen in the CBMC, suggesting that in fact we were able to see the βT protein species in human mast cells. The difficulty in visualizing βT protein could be attributed to either 1) βT being a very low abundance molecule, or 2) βT being rapidly turned over, having a short half-life and thus very little steady-state accumulation. We performed ^{35}S-methionine/cysteine metabolic labeling on U937 cells transfected with either $\alpha\beta\gamma_2$ or $\alpha\beta T\gamma_2$. FcεRIβ or βT were immunoprecipitated from lysates using their FLAG epitope tags and

samples were resolved by SDS-PAGE. After autoradiography, we observed that wild-type β was stable over the 30 min time course. In contrast, we observed that βT had an extremely short half-life. Densitometry indicated that βT had a half-life of approximately 10 min under the conditions of this experiment.

We observed herein that βT is an expressed variant protein. It is reasonable to assume, therefore, that βT may form FcεRI complexes. However, due to its variant structure and rapid turnover, we might expect that FcεRI formed of αβTγ would behave differently than their wild-type counterparts. We generated novel $\alpha\beta T\gamma_2$-expressing NIH 3T3 and U937 cell lines. We then examined the surface FcεRI expression levels in multiple clones expressing either $\alpha\gamma_2$, $\alpha\beta\gamma_2$ or $\alpha\beta T\gamma_2$ receptor isoforms. Cells were stained for surface α chain levels as described above. We observed that receptors with the wild-type β chain are significantly more expressed at the cell surface that the $\alpha\gamma_2$ isoforms. Remarkably, the presence of βT decreases surface expression of α to levels consistently below those seen with αγ transfectants. This effect was statistically significant in both the U937 and NIH 3T3 cell lines. Since βT is rapidly turned over, βT can therefore potentially negatively regulate cell surface expression of α by causing turnover of nascent receptor complexes.

To confirm this finding, we took a stable cell clone expressing αβWTγ and retransfected it in transient with either control cDNA, or the β variant cDNA. We then assessed the level of surface receptor expression as before. Transient transfection of the β variant in $\alpha\beta WT\gamma_2$ clones resulted in a significant decrease in surface FcεRI receptor expression. These results show that the βT variant acts as a dominant negative form of β for receptor expression, capable of competing with WT FcεRIβ, and of actively preventing FcεRI receptor expression.

Time (hours after retransfection)	control DNA	βT cDNA	p value βT versus control DNA
8 (n=1)	44	42	
16 (n=3)	42.3 ± 1.80	36.6 ± 2.28	p = 0.028
24 (n=4)	39.1 ± 2.93	28.8 ± 3.91	p = 0.005
40 (n=4)	37.1 ± 2.31	26.8 ± 3.61	p = 0.005

The results are expressed as mean ±SD of MFI. n = number of samples from 3 independent experiments pooled together. Unpaired t tests were used to calculate p values.

Example 4: *The βT FcεRIβ variant acts by preventing α maturation*

We also compared the intracellular trafficking of the α chain in the absence of β, in the presence of WT FcεRIβ, and in the presence of the βT FcεRIβ variant. Intracellular trafficking of the α chain can be followed quite easily experimentally due to the fact that α is highly glycosylated, and that this glycosylation is modified during trafficking. In the ER, FcεRI is glycosylated with high manose type sugars which can be cleaved *in vitro* by treatment with the enzyme Endoglycosidase H (Endo H). During trafficking through the Golgi apparatus, these sugars are replaced by complex sugars which are resistant to the action of Endo H. These different forms of FcεRIα can be identified based on their Endo H sensitivity, and apparent molecular weight after separation by polyacrylamide gel electrophoresis (PAGE). We observed a band around 30 kD that corresponds to unglycosylated FcεRIα, a band around 46 kD that corresponds to high manose glycosylated pre-Golgi FcεRIα, and a smear around 66 kD that corresponds to mature post-Golgi FcεRIα. These characteristics can be used to interpret other experiments without performing biosynthetic labeling and EndoH digestion, but with a simple PAGE and western with an anti-FcεRIα antibody (Ab). Figure 2 shows the results of such an experiment. Three clones each of transfectants expressing either $\alpha\gamma_2$, $\alpha\beta WT\gamma_2$, or $\alpha\beta T\gamma_2$ were lysed, immunoprecipitated with an anti- FcεRIα Ab, separated by PAGE, and Western blotted with an anti-FcεRIα Ab. Ab binding was revealed by chemifluorescence and each band was quantified. For each clone the amount of mature post-Golgi FcεRIα was plotted as a function of immature ER FcεRIα. Comparison of the different types of transfectants shows that for a given amount of mature post-Golgi FcεRIα, $\alpha\beta WT\gamma_2$ clones have much less immature ER FcεRIα than $\alpha\gamma_2$, and even less than $\alpha\beta T\gamma_2$. This shows that the presence of the FcεRIβ variant results in an inefficient maturation of FcεRIα which is more pronounced than in the absence of WT FcεRIβ, confirming the active role of the βT FcεRIβ variant in preventing receptor expression.

Example 5: *The FcεRIβ variant is degraded by the proteasome*

We also tested whether the β variant is degraded by the proteasome. We observed that when the β variant is immunoprecipitated and revealed by Western blotting, it is barely detectable in transfectants, even though the amount of β variant protein made by the cell is probably large compared to endogenous proteins since transcription of the transfected cDNA is controlled by a strong viral promoter. By contrast, when cells are incubated before lysis

with a proteasome inhibitor (calpain inhibitor I), the β variant is easily detected. We conclude that at least one degradation pathway utilized by the β variant involves the proteasome.

In summary, we have described 1) the identification of a novel FcεRIβ splice variant, βT, 2) that βT is a translated variant protein with the C-terminal transmembrane/cytoplasmic domains of β removed and replaced with 16 amino acids derived from intronic sequence, 3) that βT is unstable, with an apparent half-life of approximately 10 min, and 4) that αβTγ receptor isoforms are very inefficient at attaining the cell surface, in comparison with either αβγ or αγ complexes. These data therefore support that βT is an expressed negative regulator of FcεRI trafficking to the cell surface, a function that would form a stark contrast with the positive regulatory function of the wild-type β molecule. Although not wanting to be bound by a particular hypothesis, we believe that the rapid turnover of βT represents its targeting for degradation.

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All references disclosed herein are incorporated by reference in their entirety.

What is claimed is presented below and is followed by an Abstract and a Sequence Listing.

We claim: